**A Nectin-4 Targeted TLR9 Agonist Antibody Conjugate Induces Robust Immune Cell Activation and Anti-Tumor Responses**

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**Introduction**

- Novel therapies engaging both innate and adaptive immune response may produce more robust and durable anti-cancer immunity [1].
- Activation of Toll-Like Receptor 9 (TLR9) by unmethylated CpG oligodeoxynucleotides (CpG-ODNs) promotes innate inflammatory responses and the induction of adaptive immunity [2-3]. Several intramuscularly injected CpG-ODNs demonstrated clinical responses in melanoma patients [4]. We developed the Toll-Like Receptor Agonist Antibody Conjugate (TRAAC) comprised of an optimized CpG-ODN (T-CPG) conjugated to a novel Nectin-4-targeting antibody for systemic administration that enables delivery of a potent TLR9 agonist to the tumor microenvironment (TME).
- Nectin-4 is a cancer-associated antigen over-expressed in many solid tumors with limited expression in normal tissues. Additionally, Nectin-4 over-expression correlates with poor prognosis [5].
- We present preclinical data demonstrating that Nectin-4 TRAAC triggers TLR9 signaling, induces myeloid and dendritic cell activation, cancer cell phagocytosis, pro-inflammatory cytokine production and lymphocyte activation, altogether resulting in potent anti-tumor efficacy.

**Fig. 1:** Nectin-4 TRAAC, a Toll-like Receptor Agonist Antibody Conjugate designed for systemic and tumor-targeted delivery of T-CPG, an optimized TLR9 agonist

**Experimental Results**

**Fig. 2:** Anti-Nectin-4 antibody displays species cross-reactivity and self-associate binding to Nectin-4

**Fig. 3:** Nectin-4 TRAAC triggers TLR9 signaling and elicits pro-inflammatory cytokine production in human PBMCs co-cultured with Nectin-4+ cancer cells

**Fig. 4:** Nectin-4 TRAAC induces monocyte- and macrophage-mediated phagocytosis of Nectin-4+ cancer cells

**Fig. 5:** Human PBMCs from 10 healthy donors were co-cultured with Nectin-4 expressing cancer cells and left untreated or incubated with unconjugated Nectin-4 antibody or Nectin-4 TRAAC. After 14-hour incubation, the expression levels of cell surface marker indices of cell activation were determined flow cytometrically (A), CpG-ODN (B), and pro-inflammatory cytokine secretion (C).

**Fig. 6:** Nectin-4 TRAAC monotherapy exhibits potent efficacy in multiple syngeneic tumor models including checkpoint inhibitor (CPI) refractory model, EM6

**Fig. 7:** Nectin-4 TRAAC monotherapy and combination treatment efficacy in a tumor model with low Nectin-4 expression levels

**Summary and Conclusions**

- We generated high-affinity, species cross-reactive, antibodies specifically targeting Nectin-4. The Nectin-4 antibodies were conjugated with our optimized TLR9 agonist to generate Nectin-4 TRAAC.
- Nectin-4 TRAAC targets both innate and adaptive immune cells via Fcy receptor engagement leading to robust pro-inflammatory activity in cancer cell PBMC co-cultures.
- Nectin-4 TRAAC promotes phagocytosis of Nectin-4+expressing cancer cells.
- Systemic administration of single agent Nectin-4 antibody conjugated to T-CPG provides clinical efficacy in murine models with various cancer types, including those with refractory checkpoint inhibitors and tumors with low Nectin-4 expression levels.
- The preclinical data of Nectin-4 TRAAC supports its development for solid tumor malignancies that express Nectin-4.